# DECLARATION

I, Jun HAYASHI of c/o The Patent Corporate Body ARUGA PATENT OFFICE, 3-6, Nihonbashiningyocho 1-chome, Chuo-ku, Tokyo 103-0013 Japan do solemnly and sincerely declare that I well understand both Japanese and English languages and that I believe the attached English version is a true and complete translation of the Japanese Patent Application No. 11-259057 filed on September 13, 1999 in the name of Pola Chemical Industries, Inc..

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Jun HAYASHI

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【Inventor】
【Domicile or Residence】 2-14, Nijino-oka 3-chome, Izumi-ku, Sendai-shi, Miyagi
【Name】 Yoshihiro TAKAI

【Inventor】
【Domicile or Residence】 9-14, Miyagidai 3-chome, Aoba-ku, Sendai-shi, Miyagi
【Name】 Tatsuo IDO

【Inventor】
【Domicile or Residence】 c/o Pola Chemical Industries, Inc. Yokohama Kenkyusho, 27-1, Takashimadai, Kanagawa-ku, Yokohama-shi, Kanagawa
【Name】 Michihiko TSUJITANI

【Applicant for Patent】
【Identification Number】 399047404
【Name】 Yoshihiro TAKAI

【Applicant for Patent】
【Identification Number】 399047415
【Name】 Tatsuo IDO

【Applicant for Patent】
【Identification Number】 000113470
【Name】 POLA CHEMICAL INDUSTRIES, INC.

【Agent】
【Identification Number】 100068700
【Patent Attorney】
【Name】 Mitsuyuki ARUGA

Patent Application No. 11-259057

【Authorized Agent】
【Identification Number】 100077562
【Patent Attorney】
【Name】 Toshio TAKANO

【Authorized Agent】
【Identification Number】 100096736
【Patent Attorney】
【Name】 Toshio NAKAJIMA

【Authorized Agent】
【Identification Number】 100101317
【Patent Attorney】
【Name】 Hiromi MATOBA

【Authorized Agent】
【Identification Number】 100106909
【Patent Attorney】
【Name】 Sumio TANAI

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【Document Name】 Specification 1
【Document Name】 Drawing 1
【Document Name】 Abstract 1

【Request of Identification of data】 Requested

[Ref. No.] P03871109

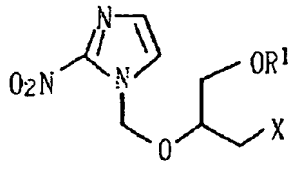
[Document Name] Specification

[Title of the Invention] Diagnostic Imaging Agent

[Claims]

[Claim 1] A diagnostic imaging agent comprising, as an active ingredient, a nitroimidazole derivative represented by the following formula (1):

[F1]



(1)

[wherein $R^1$ represents a hydrogen atom or a C1-C4 alkanoyl group; and X represents a fluorine atom or an isotope thereof].

[Claim 2] A diagnostic imaging agent according to claim 1, which is used for imaging an ischemic site.

[Claim 3] A diagnostic imaging agent according to claim 1 or 2, wherein X is [18-F].

[Detailed Description of the Invention]

[0001]

[Technical Field to Which the Invention Pertains]

The present invention relates to a diagnostic imaging agent which is useful for imaging an ischemic site.

[0002]

[Conventional Art]

In recent years, dietary lifestyle of the Japanese people has become Europeanized or Americanized, and in accordance with this trend, patients suffering diseases of

the circulatory system, such as hyperlipidemia, angina pectoris, and myocardial infarction, have been drastically increasing. Such a disease may cause damage to nutrition-supplying organs of the body, such as the heart and blood vessels, and may be life threatening, depending on the progress of the disease. Therefore, the site of the disease must be determined at early stages of the disease and the disease must be subjected to appropriate treatment.

In ischemic diseases, peripheral tissues of ischemic sites are destroyed by active oxygen or similar substances, and thus it is important not only to find the presence of vasoconstriction sites or heart valve disorder, but also to determine ischemic sites which are generated due to lack of blood flow. Briefly, damaged tissues at such ischemic sites, as well as vasoconstriction sites and cardiac dysfunction, may be life threatening.

[0003]

In recent years, diseases of the circulatory system have been reliably diagnosed, and the sites of the diseases have been precisely determined through angiography, electrocardiogram, load electrocardiogram, 24-hour monitoring, or similar means. However, even when such a method is employed, ischemic sites or tissues cannot be detected directly, and biopsy has been the main means for detecting damage derived from ischemia. Therefore, there has been demand for means to determine ischemic sites conveniently and reliably.

[0004]

[Problems to be Solved by the Invention]

Thus, an object of the present invention is to provide a means for identifying, in a non-invasive manner, ischemic sites of the circulatory system, which occur in association with circulatory diseases.

[0005]

[Means for Solving the Problems]

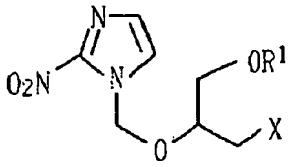
In view of the foregoing, the present inventors have performed extensive studies, and have found that a specific nitroimidazole derivative is selectively directed to ischemic sites of circulatory organs; and that when the derivative is employed as a contrast medium in diagnostic imaging, imaging of the ischemic sites can be attained. The present invention has been accomplished on the basis of these findings.

[0006]

Accordingly, the present invention provides a diagnostic imaging agent comprising, as an active ingredient, a nitroimidazole derivative represented by the following formula (1):

[0007]

[F2]



( ] )

[0008]

[wherein $R^1$ represents a hydrogen atom or a C1-C4 alkanoyl

group, and X represents a fluorine atom or an isotope thereof].

[0009]

[Modes for Carrying Out the Invention]

A nitroimidazole derivative represented by formula (1) of the present invention is a novel compound, and a fluorine atom or an isotope thereof represented by X in the formula is a stable isotope of fluorine [19-F] or a radioisotope of fluorine [18-F]. When X is the radioisotope [18-F], the location of the derivative of the present invention in the body can be visualized through positron emission tomography (PET). When X is the non-radioactive stable isotope [19-F], the location of the derivative in the body can be visualized through MRI or a similar means. A derivative in which not all fluorine atoms are radioisotopes still plays an important role in imaging, because it can serve as a diluting agent for the radioisotope-bearing compound.

[0010]

A C1-C4 alkanoyl group represented by $R^1$ may be an acetyl group, a propionyl group, a butyryl group, or an isobutyryl group, with an acetyl group being particularly preferred.

In the present invention, $R^1$ is particularly preferably a hydrogen atom, in consideration of control of imaging being enabled.

[0011]

Examples of particularly preferred compounds of the

present invention include 1-[2-fluoro([18-F] or [19-F])-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole and 1-[1-acetoxymethyl-2-fluoro([18-F] or [19-F])ethoxy]methyl-2-nitroimidazole.

[0012]

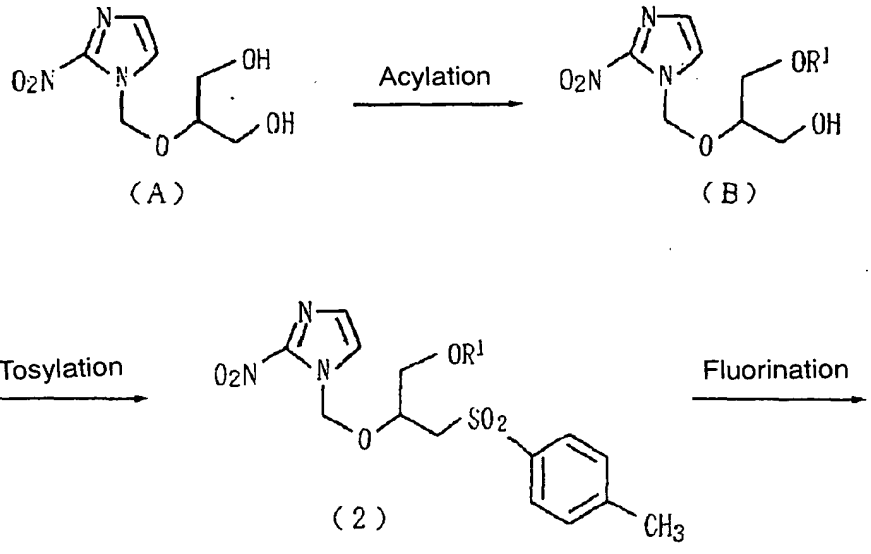
The compound (1) of the present invention contains an asymmetric carbon atom, and thus there exist stereoisomers of the compound which are derived from the asymmetry. The present invention encompasses the stereoisomers, and the streoisomers may be employed singly or in combination.

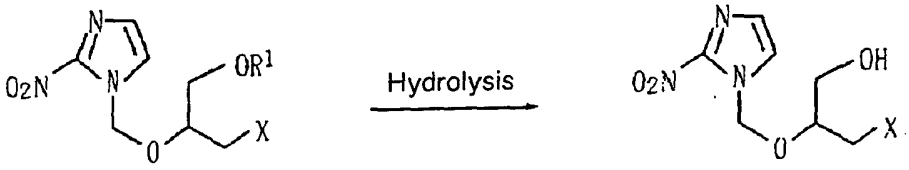
[0013]

The nitroimidazole derivative (1) of the present invention may be produced through, for example, the following steps:

[0014]

[F3]






(1-A) (1-B)

[0015]

[wherein $R^1$ and X have the same meanings as described above].

[0016]

Briefly, a hydroxy form (A) is acylated to produce an ester form (B), and then the ester form is tosylated to produce a novel intermediate, tosyl form (2). Subsequently, the tosyl form is fluorinated, producing a nitroimidazole derivative (1-A) of the present invention in which $R^1$ is an alkanoyl group. If desired, the derivative (1-A) may be subjected to hydrolysis, to thereby obtain a nitroimidazole derivative (1-B) of the present invention, in which $R^1$ is hydrogen.

[0017]

Acylation may be carried out through a customary acylation scheme; for example, may be carried out by use of an acid halide in a solvent at -30 to 100°C for one to five hours in the presence or absence of an inorganic base, an organic base, or an organometallic compound. In acylation, the inorganic base may be potassium hydroxide, sodium carbonate, or cesium carbonate; the organic base may be pyridine, 4-dimethylaminopyridine, picoline, N,N-dimethylaniline, N-methylmorpholine, dimethylamine,

triethylamine, or 1,8-diazabicyclo[5.4.0]undecene (DBU); and the organometallic compound may be dibutyl tin oxide. Examples of solvents which may be employed include halogenated hydrocarbons such as methylene chloride, chloroform, carbon tetrachloride, and chlorobenzene; aromatic hydrocarbons such as benzene and toluene; ethers such as tetrahydrofuran, diethyl ether, and dioxane; ketones such as acetone and methyl ethyl ketone; non-protonic polar solvents such as acetonitrile and N,N-dimethylformamide; and ethyl acetate.

[0018]

Tosylation may be carried out through a customary method; for example, may be carried out by use of 2-3 mol of tosyl halide (e.g., tosyl chloride) with respect to 1 mol of a material compound in the presence of a base such as triethylamine in an organic solvent such as methylene chloride, acetonitrile, dimethylformamide, or pyridine at 0-100°C for one to five hours.

[0019]

Fluorination may be carried out in an inert solvent by use of crown ether serving as a catalyst and by use of a fluorination agent such as an alkali metal fluoride (e.g., sodium fluoride, potassium fluoride, or cesium fluoride) or a tetraammonium fluoride (e.g., tetrabutylammonium fluoride). An inert solvent is preferably a halogen solvent, an ether solvent, a hydrocarbon solvent, a polar solvent, or a solvent mixture thereof. Fluorination is usually carried out at

about 70-130°C, and preferably at 100-120°C in the case in which DMF is employed as a solvent.

[0020]

When a [18-F] fluoride (e.g., [18-F]K) is employed as a fluorination agent, fluorination is preferably carried out by use, for example, of cryptofix 2.2.2 serving as a phase transfer catalyst. In this connection, a [18-F] fluoride source can be obtained by using enriched $H_2{}^{18}O$ as a target, trapping an aqueous solution of $^{18}F$ obtained from $^{18}O$ (P.n.) with an anion exchange resin, and eluting the solution with an aqueous solution of potassium carbonate.

[0021]

Hydrolysis may be carried out in the presence of an inorganic base in a solvent at 0°C to 100°C for one to five minutes. The inorganic base may be potassium hydroxide, sodium hydroxide, potassium carbonate, sodium carbonate, or cesium carbonate. The solvent may be water; an alcohol such as methanol, ethanol, or propanol; an ether such as tetrahydrofuran, diethyl ether, or dioxane; or a ketone such as acetone or methyl ethyl ketone.

[0022]

When the thus-produced nitroimidazole derivative (1) of the present invention is administered to a living organism, as shown in the below-described Test Example, the derivative recognizes ischemic sites, and is rapidly directed thereto. Therefore, the derivative is useful as an imaging agent for image diagnoses, and when it is employed together with an

apparatus for diagnostic imaging such as MRI, the location at which ischemic sites exist can be detected and the severity of ischemia can be measured.

[0023]

The nitroimidazole derivative (1) of the present invention may be mixed with a pharmaceutically acceptable additive, to thereby yield a diagnostic imaging agent. Examples of additives include pharmaceutically acceptable isotonic agents, emulsifying and dispersing agents, excipients, binders, coating agents, stabilizers, sugars such as mannitol, and freeze-dry-aiding agents such as amino acids.

[0024]

The diagnostic imaging agent of the present invention may be administered orally or parenterally; for example, through generally employed means such as intravenous injection. In particular, a nitroimidazole derivative (1) of the present invention having a hydrogen atom as $R^1$ is water-soluble and tends to be directed to and accumulated in ischemic smooth muscle cells, and thus the agent may be administered in the dosage form of injection. Meanwhile, the nitroimidazole derivative (1) comprising an alkanoyl group as $R^1$ may be administered orally as a prodrug in the dosage form of enteric-coated drug, since the alkanoyl group easily undergoes dealkanoylation in a living organism.

The derivative of the present invention is preferably administered about 2-3 hours before radiography or MRI.

[0025]

The dose of the diagnostic imaging agent of the present invention is determined in consideration of various conditions such as the weight, age, and sex of a patient, and the imaging apparatus employed. When the diagnostic imaging agent is employed in MRI, the dose is preferably 0.1-10 g per person. When the agent is employed in PET, at least 0.01% or thereabouts of the agent are preferably replaced by a radioisotope of fluorine. In PET, a level of 1 ng to 1 μg of the agent can be detected, and thus the dose of the agent may be reduced futher.

[0026]

[Examples]

The present invention will next be described in more detail by way of examples.

Referential Example 1

[0027]

Synthesis of 1-[1-acetoxymethyl-2-(hydroxy)ethoxy]methyl-2-nitroimidazole

2-Nitroimidazole was subjected to trimethylsilylation by use of hexamethyldisilazane in acetonitrile, and the resultant compound and 2-acetoxymethoxy-1,3-diacetoxypropane were subjected to condensation by use of stannic chloride serving as a catalyst. The resultant product was deprotected by use of methanolic ammonia, to thereby obtain 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole. The thus-obtained 1-[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (0.5 g) was refluxed for two hours together

with dibutyl tin oxide (0.6 g) in anhydrous toluene in the presence of molecular sieves having a pore size of 4 Å. The solvent was removed under reduced pressure, and anhydrous methylene chloride (16 mL) and anhydrous tetrahydrofuran (4 mL) were added to the residue. The resultant mixture was cooled to 0°C, and acetyl chloride (171 mg) was added to the mixture, and then the mixture was stirred for 30 minutes. To the resultant reaction mixture, a sodium phosphate buffer having a pH of 7.1 (10 mL) was added, and the resultant mixture was subjected to filtration. The resultant residue was subjected to extraction with chloroform (10 mL × 3), the thus-obtained extract was mixed with the filtrate, and the mixture was separated, thereby obtaining an organic layer. The organic layer was dried over sodium sulfate, and then fractionated and purified through silica gel chromatography, to thereby yield the title compound, 1-[1-acetoxymethyl-2-(hydroxy)ethoxy]methyl-2-nitroimidazole (265 mg).

[0028]

Referential Example 2

Synthesis of 1-[2-(toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole

1-[1-Acetoxymethyl-2-(hydroxy)ethoxy]methyl-2-nitroimidazole (117 mg) was placed in a flask together with anhydrous pyridine, toluenesulfonyl chloride (252 mg) was added to the flask, and the resultant mixture was stirred at room temperature for five hours. The reaction mixture was subjected to extraction with ethyl acetate (30 mL), and the

resultant extract was partitioned and washed with water (30 mL × 2). The resultant organic layer was dried over sodium sulfate, concentrated under reduced pressure, and purified through silica gel column chromatography, to thereby yield the title compound 1 (90.2 mg).

[0029]

$^1$H−NMR ($CD_3CN$) : δ ppm

1.88 (s, 3H)、2.44 (s, 3H)、3.96～4.11 (m, 4H)、5.68、5.78 ( AB pattern ; J=1.0Hz、2H)、7.11 (d, J=8.5Hz, 1H)、7.39 (d, J=1.0Hz, 1H)、7.42 (d, J=8.5Hz, 1H)、7.73 (d, J=8.5Hz, 1H)

$^{13}$C−NMR ($CD_3CN$) : δ ppm

20.7、21.6、63.1、69.8、75.6、78.5、127.2、128.8、131.1、171.1

Mass spectrum : 413 ($M^+$)

[0030]

Example 1

Synthesis of 1-[1-acetoxymethyl-2-fluoroethoxy]methyl-2-nitroimidazole (compound 1)

Acetonitrile (10 mL) was mixed with water (1 mL), and potassium fluoride (33.8 mg) and 18-crown-6 (80 mg) were added to the solution. After the solution was dried under reduced pressure, compound 1 (89.2 mg) in anhydrous dimethylformamide (10 mL) was added to the dried solution, and the resultant mixture was heated at 110°C for eight hours. Ethyl acetate (20 mL) was added to the resultant reaction mixture, and then the mixture was washed with water (20 mL).

The water layer was subjected to extraction with ethyl acetate (20 mL × 2), the resultant extract was mixed with the organic layer, and the resultant mixture was dried under reduced pressure. The dried product was purified through separable high performance chromatography, to thereby yield the title compound 2 (16.2 mg).

[0031]

$^1$H−NMR ($CD_3CN$) : δ ppm

1.94 (s, 3H)、3.98～4.14 (m, 3H)、4.38～4.58 (m, 2H)、5.79、5.86 (AB pattern ; J＝1.2Hz、2H)、7.13 (d, J＝1.2Hz, 1H)、7.51 (d, J＝1.1Hz, 1H)

$^{13}$C−NMR ($CD_3CN$) : δ ppm

20.8、62.9、76.7、78.8、83.6、127.2、128.8、171.3

Mass spectrum : 261 ($M^+$)

[0032]

Example 2

Synthesis of 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 2)

A 50 V/V% aqueous solution (2 mL) of ethanol containing 0.05N sodium hydroxide was added to compound 1 (18 mg) prepared in Example 1, and the mixture was stirred at 40°C for 1.5 minutes. The resultant reaction mixture was added to an ion exchange column to remove sodium cations. Thereafter, the resultant mixture was concentrated under reduced pressure, and then purified through separable high performance

chromatography, to thereby yield the title compound 3 (10.3 mg).

[0033]

$^1$H−NMR ($CD_3CN$) : $\delta$ ppm

3.01 (br, 1H)、3.49～3.53 (m, 2H)、4.32～4.54 (m, 2H)、5.83、5.85 (AB pattern ; J=10.8Hz、2H)、7.11 (d, J=1.1Hz, 1H)、7.51 (d, J=1.1Hz, 1H)

$^{13}$C−NMR ($CD_3CN$) : $\delta$ ppm

61.1、79.1、79.9、84.1、127.0、128.8

Mass spectrum : 220.07 ($M^{+1}$)

[0034]

Example 3

Synthesis of 1-[2-fluoro[18-F]-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 3)

In a manner similar to those described in Examples 1 and 2, 1-[2-(toluene-4-sulfoxy)-1-(acetoxymethyl)ethoxy]methyl-2-nitroimidazole was reacted with K[18-F] (prepared by use of a cyclotron HW-12, 3.7 GBq) by use of cryptofix 2.2.2 serving as a phase transfer catalyst, to thereby yield the title compound 4 (150 MBq). Through high performance liquid chromatography, this compound was found to have elution properties which are the same as those of 1-[2-fluoro-1-(hydroxymethyl)ethoxy]methyl-2-nitroimidazole (compound 3) of Example 3. Therefore, this compound was found to be a compound in which a fluorine atom of compound 3 was replaced by [18-F].

[0035]

Test Example

Imaging of an ischemic site of the heart was carried out by use of compound 3 prepared in Example 3.

Briefly, a male Donryu rat was anesthetized with pentobarbital, and the respiration of the rat was regulated by use of a respirator. The left chest of the rat was opened at the position between the seventh and eighth sterna, and the pericardium was incised for exposure of the heart. The left anterior descending artery stem of the coronary artery was ligated in order to induce ischemia. Separately, compound 3 was diluted with compound 2 so as to attain a radiation intensity of 150 MBq. The thus-diluted compound 3 was administered intravenously to the rat 15 minutes after completion of ligation. The heart was extirpated 40 minutes after administration of the compound 3, a frozen section of the heart was prepared, and the section was brought into contact with an imaging plate, whereby an autoradiogram as shown in Fig. 1 was obtained.

The autoradiogram revealed that the diagnostic imaging agent of the present invention exists at relatively high concentration at a muscle tissue site in the vicinity of the left ventricle, at which ischemia is usually generated by such ligation, and thus the agent appropriately images an ischemic site.

[0036]

[Effects of the Invention]

Use of the diagnostic imaging agent of the present invention enables imaging of ischemic sites of circulatory organs caused by circulatory organ diseases, and thus can provide information about the locations of ischemic sites and the severity of ischemia. Therefore, the diagnostic imaging agent greatly contributes to selection of appropriate treatment, due to its capability of non-invasive identification of ischemic sites.

[Brief Description of the Drawing]

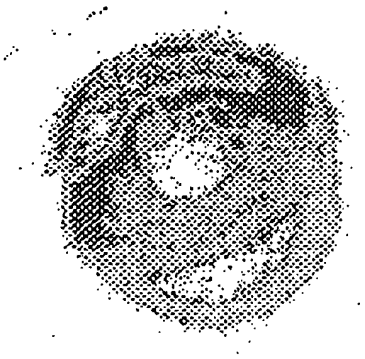
[Fig. 1]

An autoradiogram of an ischemic heart, which is obtained by use of the diagnostic imaging agent of the present invention.

[Ref. No.] P03871109

[Document Name] Drawing

[Fig. 1]



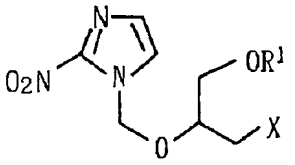
[Ref. No.] P03871109

[Document Name] Abstract

[Abstract]

[Means for Solution] A diagnostic imaging agent containing, as an active ingredient, a nitroimidazole derivative represented by the following formula (1):

[F1]



(1)

[wherein $R^1$ represents a hydrogen atom or a C1-C4 alkanoyl group; and X represents a fluorine atom or an isotope thereof].

[Effects] The present invention enables imaging of ischemic sites which are caused in association with circulatory diseases, providing information about the locations of ischemic sites and the severity of ischemia. Therefore, the diagnostic imaging agent greatly contributes to selection of appropriate treatment and to improvement of efficacy of the treatment.

[Selected Drawing] None